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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Gardner *et al.*

Date: April 14, 2003

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Docket No.: 033948-0102

Filed: June 8, 2001

Examiner: J. Angell

For: **MAMMALIAN GAMETE AND EMBRYO CULTURE MEDIA SUPPLEMENT AND METHOD FOR USING SAME**

DECLARATION OF DAVID K. GARDNER

I, David K. Gardner, do hereby declare and state as follows:

I have been involved in the field of embryology research for more than 19 years. I have been employed at the Colorado Center for Reproductive Medicine (CCRM) for the last 5 years. I presently hold the position of Scientific Director. My responsibilities at CCRM include running the Clinical In Vitro Fertilization laboratory as well as an Embryology Research Program.

I received a Bachelors of Science in Biology from the University of York (UK) in 1984 and a Ph.D. in Embryology from the University of York in 1987.

My curriculum vitae is attached hereto as exhibit A.

I have reviewed the disclosure of U.S. Patent Number 6,153,582 issued to Skelnick (hereinafter "the '582 patent"). The '582 patent teaches a serum-free medium suitable for use in preserving corneal tissue. The medium of the '582 patent includes components selected from sixteen different groups, as outlined at column 3, line 52 through column 4, line 55. The sixteen groups are labeled (a) through (p) in claim 1 of the '582 patent. Components of group (i) are ATP and energy precursors. Examples of group (i) components are provided in column 4, lines 14-17 of the '582 patent. Components of group (k) are co-enzymes and enzyme supplements. Examples of group (k) components are provided in column 4, lines 27-29 of the '582 patent. Components of group (l) are nucleotide precursors. Examples of group (l) components are provided in column 4, lines 30-33 of the '582 patent. Components of group (o) are trace minerals and trace elements. Examples of group (o) components are provided in column 4, lines 39-44 of the '582 patent. Based on my knowledge of embryology, it is my opinion that a culture medium that includes components (i), (k), (l), and (o) of the medium disclosed in the '582 patent would be inhibitory to any embryos cultured in that medium. This conclusion is supported by the analysis discussed directly below.

I performed an analysis of the serum-free solution for corneal storage made substantially as described in the '582 patent for use as an embryo culture medium. The solution used in my analysis (hereinafter "the Skelnick medium") was prepared using one component

from each of the sixteen groups that make up the corneal storage medium described in column 3, line 51 through column 4, line 55 of the '582 patent. The specific components and the relative amounts of the components were selected with the objective of providing a medium having the highest chance of promoting embryo viability. The sixteen groups, which are labeled groups (a) through (p) in claim 1 of the '582 patent are: (a) an aqueous nutrient and electrolyte solution; (b) a glycosaminoglycan; (c) a deturgescent agent; (d) an energy source; (e) a buffer system; (f) an antioxidant; (g) membrane stabilizing agents; (h) antibiotic and antimycotic agents; (i) ATP/energy precursors; (j) nutrient cell supplements; (k) coenzymes and enzyme supplements; (l) a nucleotide precursor; (m) a hormonal supplement; (n) non-essential amino acids; (o) trace minerals/trace elements; and, (p) a growth factor. The specific component selected from each group and the amount of each component used in the Skelnick medium are shown in Table 1 below.

Table 1.

Group	Component	Amount
A	Base Medium = MEM (Minimal Essential Medium)	
B	Hyaluronic Acid	0.125 mg/ml
C	Albumin	5 mg/ml
D	Pyruvate	0.1 mM
E	Bicarbonate	25.0 mM
F	Ascorbic Acid	0.1 mM
G	Transferin	0.1 mg/ml
H	Penicillin	0.6 mg/ml
I	NAD	100 μM
J	Alynyl-glutamine	0.5 mM
K	Co-enzyme A	100 μM
L	2-Deoxyadenosine	0.1 μM
M	β-estradiol	0.01 pg/ml
N	Glycine	100 μM
O	NaF	1.0 ng/ml
p	PDGF-AA	1.0 ng/ml

A sequential culture system was used as the control media. The formulations of the G1.2/G2.2 control media are listed in the reference: Gardner DK and Lane M (2002) Culture media for the human embryo. In *Biotechnology of Human Reproduction*. Ed. A Revelli, I Tur-Kaspa, JG Holte, M Massobrio. Parthenon Press. pp 181-199 (attached). These media were supplemented with 0.125mg/ml hyaluronan.

The Skelnick medium was analyzed for use as an embryo culture medium according to the following procedure. Embryos from F1 hybrid females (C57Bl6xCBA) were collected from the tract at the zygote (1-cell) stage and cultured. Embryos were cultured in the

same dish in 20µl drops of medium under oil at 37 °C under an atmosphere of 6% CO_2:5% O_2:89% N_2. Development to the 2-cell stage was assessed after 24h of culture and development to the blastocyst stage determined after 96h of culture. The results are summarized in Table 2 below.

Table.

Medium	Number of embryos	Percentage 2-cell development	Percentage blastocyst development
G1.2/G2.2	42	100%	95.2%
Skelnick Medium	42	0%	0%

All embryos cultured in the Skelnick medium experienced developmental arrest at the 1-cell stage and degenerated. None of the embryos cultured in the Skelnick medium survived to the 2-cell stage.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 14 April 2008 Signature: D. K. Gardner

EXHIBIT A

DR. DAVID K. GARDNER

PERSONAL INFORMATION

- Born on 12 August 1963
- Nationality: Australian/British
- Marital Status: Married, four children
- Business Address: Colorado Center for Reproductive Medicine
  799 East Hampden Ave., Suite 520
  Englewood (Denver), Colorado 80110
  United States of America
- Telephone: (303) 788 4868 Fax: (303) 788 4438
- Email: dgardner@colocrm.com

CURRENT POSITIONS HELD

- **Scientific Director**, Colorado Center for Reproductive Medicine, Englewood.
- **Scientific Director,** Houston IVF, Texas.
- **Adjunct Professor**, Department of Physiology, Colorado State University.
- **Visiting Professor,** University of Zhongshan, China

EDUCATION

1981-1984	**Biology Department, University of York, U.K.**	B.Sc. (Hons) Biology
1984-1987	**Biology Department, University of York**	Ph.D.

CAREER ACHIEVEMENTS

Oct 1987 - July 1989	**Research Fellow**, Department of Biology, University of York, UK. Laboratory of Dr. Henry J. Leese.
Apr 1988 - July 1988	**Research Fellow in Molecular and Cellular Physiology** Laboratory of Human Reproduction and Reproductive Biology Harvard University, Boston, United States of America Laboratory of Professor John D. Biggers.
Aug 1989 to 1997	**Head**, Embryo Physiology Laboratory, Centre for Early Human Development.
Aug 1989 - Jan 1991	**Research Officer & Deputy Head of Embryology & Fertility Group**. Centre for Early Human Development, Monash University, Australia. Director: Professor Alan O. Trounson.
Feb 1991 - Dec 1991	**Senior Research Officer & Deputy Head of Embryology & Fertility Group,** Centre for Early Human Development.
Sept 1991 – Dec 1999	**Honorary Lecturer** in Obstetrics & Gynaecology, Monash University.
Jan 1991 – Sept 1997	**Clinical Embryologist**, Monash IVF (part time).
Jan 1992 - Aug 1997	**Research Fellow & Deputy Head of Laboratories of Human & Animal Reproductive Biology**, Institute of Reproduction & Development, Monash University.

Mar 1994 - Aug 1997	**Senior Scientist**, Institute of Reproduction and Development.
Jan 1994 – Dec 1999	**Research Associate**, Department of Zoology, University of Melbourne.
Sept 1997 – Sept 1999	**Director of Research and Development**, Colorado Center for Reproductive Medicine, Englewood, Colorado Medical Director: Dr. William B. Schoolcraft
Oct 1999 – Present	**Scientific Director**, Colorado Center for Reproductive Medicine
April 2002 – Present	**Scientific Director**, Houston IVF

AWARDS

1984-1987	Science and Engineering Research Council Scholarship (UK)
1985/1986	K.M.Stott Prize, University of York, for Excelling in Scientific Research
1988	Agricultural and Food Research Council Travelling Fellowship (UK)
1991	Junior Scientist Award: Australian Society for Reproductive Biology
1994-1997	Australian Research Council Fellowship
1997	SART Prize Paper, American Society for Reproductive Medicine
2002	Visiting Professor, University of Zhongshan, China

STUDENT SUPERVISION

- Michelle Lane, Ph.D. student. Currently Senior Scientist at the Colorado Center for Reproductive Medicine.
- Rebecca Spindler, Ph.D. student, Melbourne University, (co-supervisor with Professor Renfree). Currently Research Fellow with Dr David Wild, Front Royal, Washington DC.
- Tracey Steeves, Ph.D. student. Currently Laboratory Director, Monash IVF, Clayton, Australia.
- Dr Frank Tsai, Masters student. Currently Director of Embryology at St. Joseph Hospital, Kaoshuing, Taiwan.
- Co-Supervisor of one Masters student and one Honours student.
- Supervisor of seven Honours students.

EDITORIAL BOARD

- Molecular Human Reproduction (1998 – 2000)
- Molecular Reproduction and Development (2000 – present)
- Israeli Journal of Obstetrics and Gynecology (2000 – present)

REVIEWER FOR THE FOLLOWING INTERNATIONAL JOURNALS

- *Human Reproduction*
- *Biology of Reproduction*
- *Molecular Reproduction and Development*
- *Theriogenology*
- *Fertility and Sterility*
- *Journal of Reproduction and Fertility*
- *Reproduction Fertility and Development*

PROFESSIONAL SOCIETIES

- American Society for Reproductive Medicine
- Australian Society for Reproductive Biology
- Society for the Study of Fertility (U.K)
- European Society for Human Reproduction and Embryology
- Society for the Study of Reproduction (U.S.A.)
- International Embryo Transfer Society

COMMITTEES

- Newsletter Editor & Committee Member of the Australian Society for Reproductive Biology from 1993 to 1996.
- Co-organizer of ASRB Micromanipulation Workshop, Monash University, June 1995
- Organizer of ASRB Embryo Workshop, Melbourne Zoo, September 1995
- Organizer of Serono Symposium on ART and the Human Blastocyst, Dana Point, CA, 2000

GRANTS AWARDED

1984	**SERC (UK) PhD Scholarship.**	$15,000
1988	**AFRC (UK) Travelling Fellowship.**	$ 8,000
1990	**NH&MRC Equipment Grant** for quantitative fluorescence microscope (with Professor Alan Trounson).	$53,000
1990	**Monash University** to study the viability of the preimplantation mouse embryo.	$ 4,000
1991	**Australian Research Council (ARC) Small Grant** to study the culture of preimplantation embryos of domestic animals.	$10,000
1992-94	**Dairy Research and Development Corporation** Development of culture systems for embryos of domestic animals.	$170,000
1993	**ARC Small Grant** (with Professor Renfree and Dr Shaw, Melbourne University) Embryonic diapause in the Wallaby.	$20,000
1994-98	**ARC Fellowship** Regulation of Energy Metabolism in the Preimplantation Mammalian Embryo	$286,000
1994-96	**ARC Large Grant** (with Professor Renfree and Dr Shaw, Melbourne University) Metabolic Reactivation after Embryonic Quiescence in a Marsupial	$147,000
1994	**Dairy Research and Development Corporation** (4th year extension) Development of culture systems for embryos of domestic animals.	$59,800
1995	**ARC Small Grant** Energy Production by the Mammalian Preimplantation Embryo	$11,000
1995-97	**Dairy Research and Development Corporation** Culture, cryopreservation and viability determination of bovine embryos	$210,000
1995	**NH&MRC Equipment Grant** for Confocal Microscope (with Professor Alan Trounson)	$50,000
1995	**Ramaciotti Equipment Grant** for Confocal Microscope (with Professor Alan Trounson)	$80,000
1996-98	**ARC Large Grant** (with Dr D Williams, University of Melbourne) Regulation of intracellular pH in preimplantation mammalian embryos	$183,000
1997	**IVF Friends, Melbourne** Role of amino acids in regulating embryo viability	$4,500
2000	**Organon** for Fluorescence microscope	$25,000
2001	**HealthONE Alliance** Induction of aberrant fetal growth at the preimplantation stage of mammalian embryo development	$40,000
2002	**HealthONE Alliance** Cryopreservation of mammalian oocytes	$35,000

PUBLICATIONS

Books

1. *Handbook of In Vitro Fertilization.*
   Eds. AO Trounson and **DK Gardner**. CRC Press, Boca Raton. 1993.

2. *Handbook of In Vitro Fertilization* (Second Edition)
   Eds. AO Trounson and **DK Gardner**. CRC Press, Boca Raton. 1999.

3. *Seminars in Reproductive Medicine: Novel Approaches to Assisted Reproduction: In Vitro Maturation of Gametes and Embryos.*
   Eds. **DK Gardner** and Z Rosenwaks. Thieme, New York. 2000.

4. *Textbook of Assisted Reproductive Techniques: Laboratory and Clinical Perspectives*
   Eds. **DK Gardner**, A Weissman, C Holwes and Z Shoham. Martin Dunitz Press, London. 2001.

5. *ART and the Human Blastocyst.*
   Eds. **DK Gardner** and M Lane. Springer-Verlag, New York. 2001.

6. *A Laboratory Guide to the Mammalian Embryo.*
   Eds. **DK Gardner,** M Lane and AJ Watson. Oxford University Press. 2003.

7. *Textbook of Assisted Reproductive Techniques: Laboratory and Clinical Perspectives* (Second Edition)
   Eds. **DK Gardner**, A Weissman, C Holwes and Z Shoham. Martin Dunitz Press, London. 2004.

Chapters in Books

1. Biggers JD, **Gardner DK** and Leese HJ (1989) Control of carbohydrate metabolism in preimplantation mammalian embryos. in *Regulation of Growth in Development.* Eds.IY Rosenblum and S Heyner. CRC Press, Boca Raton, pp 19-32.

2. Leese HJ, **Gardner DK**, Gott AL, Handyside AH, Hardy K, Hooper MAK, Rutherford AJ and Winston RML (1990) Non-invasive biochemical methods for assessing human embryo quality. in *Advances in Assisted Reproductive Technologies.* Ed. S Mashiach. Plenum Press, New York, pp 737-744.

3. **Gardner DK** and Lane M (1993) Embryo culture systems. in *Handbook of In Vitro Fertilization.* eds. A Trounson and DK Gardner. CRC Press, Boca Raton, pp 85-114.

4. **Gardner DK** and Leese HJ (1993) Assessment of embryo metabolism and viability. in *Handbook of In Vitro Fertilization.* eds. A Trounson and DK Gardner. CRC Press, Boca Raton, pp 195-211.

5. Sakkas D and **Gardner DK** (1993) Assessing embryo cleavage rates, viability and metabolism to detect critical stages in embryo culture. in *Implantation in Mammals.* eds. L Gianaroli, A Campana and A Trounson. Serono Symposia Publications, Raven Press, New York, pp 309-317.

6. **Gardner DK**, Lane M, Kouridakis K, and Schoolcraft WB (1997) Complex physiologically based serum-free culture media increase mammalian embryo development. in *In Vitro Fertilization and Assisted Reproduction.* Eds. V Gomel and Leung PCK. Monduzzi Editoire, Bologna, pp 187-191.

7. **Gardner DK** (1998) Embryo development and culture techniques. in *Animal Breeding: Technology for the 21st Century.* ed. J Clark. Harwood Academic Publishers, London, pp13-46.

8. **Gardner DK** and Schoolcraft WB (1998) Elimination of high order multiple gestations by blastocyst culture and transfer. in *Female Infertility Therapy: Current Practice*. Eds. Z. Shoham, C Howles and H Jacobs. Martin Dunitz, London, pp. 267-74.

9. **Gardner DK** (1998) Improving embryo culture and enhancing pregnancy rate. in *Female Infertility Therapy: Current Practice*. Eds. Z. Shoham, C Howles and H Jacobs. Martin Dunitz, London, pp. 283-99.

10. Bongso A and **Gardner DK** (1999) Embryo development. in *Handbook of In Vitro Fertilization, Second Edition*. eds. A Trounson and DK Gardner. CRC Press, Boca Raton, pp 167-180.

11. Bongso A, Sakkas D and **Gardner DK** (1999) Co-culture of embryos with somatic helper cells. in *Handbook of In Vitro Fertilization, Second Edition*. eds. A Trounson and DK Gardner. CRC Press, Boca Raton, pp 181-204.

12. **Gardner DK** and Lane M (1999) Embryo culture systems. in *Handbook of In Vitro Fertilization, Second Edition*. eds. A Trounson and DK Gardner. CRC Press, Boca Raton, pp 205-264.

13. **Gardner DK** and Leese HJ (1999) Assessment of embryo metabolism and viability. in *Handbook of In Vitro Fertilization, Second Edition*. eds. A Trounson and DK Gardner. CRC Press, Boca Raton, pp 347-372.

14. **Gardner DK** and Schoolcraft WB (1999) In-vitro culture of human blastocysts. in *Towards Reproductive Certainty: Fertility and Genetics Beyond 1999*. eds. Jansen, R. and Mortimer, D. Parthenon Press, Carnforth, pp 378-388.

15. **Gardner DK** and Lane M (2001) Embryo culture. in *Textbook of Assisted Reproductive Technology: Laboratory and Clinical Perspectives*. eds. DK Gardner, A Weissman, C Holwes and Z Shoham. Martin Dunitz Press, London. pp 203-222.

16. **Gardner DK** (2001) Improving implantation rates in IVF. In Lessey, B. (ed) *Infertility and Reprod. Med. Clinics of North America, Assisted Reproduction: Laboratory Considerations*. W. B. Saunders Company, Philadelphia.

17. Lane M and **Gardner DK** (2001) Embryo homeostasis. in *ART and the Human Blastocyst*. eds. DK Gardner and M Lane. Springer-Verlag, New York. pp. 69-90.

18. **Gardner DK** and Lane M (2001) Culture systems and blastocyst development. in *ART and the Human Blastocyst*. eds. DK Gardner and M Lane. Springer-Verlag, New York. pp. 118-43.

19. **Gardner DK** and Lane M (2002) Development of viable mammalian embryos in vitro: Evolution of sequential media. in *Principles of Cloning*. eds. J Cibelli, R Lanza, K Campbell, and MD West. Academic Press San Diego. pp. 187-213.

20. **Gardner DK** (2002) Embryo metabolism. in *Reproduccion Humana*. eds. J Remohi, A Pellicer, C Simon and J Navarro. pp 413-420.

21. **Gardner DK** and Lane M (2002) Culture media for the human embryo. In *Biotechnology of Human Reproduction*. Ed. A Revelli, I Tur-Kaspa, JG Holte, M Massobrio. Parthenon Press. pp 181-199.

22. Lane M and **Gardner DK** (2003) Preparation of gametes, in vitro maturation. In vitro fertilization and embryo recovery. In *A Laboratory Guide to the Mammalian Embryo* .eds. DK Gardner, M Lane and AJ Watson. Oxford University Press. In press.

23. **Gardner DK** and Lane M (2003) Culture of the mammalian preimplantation embryo. In vitro fertilization and embryo recovery. In *A Laboratory Guide to the Mammalian Embryo* .eds. DK Gardner, M Lane and AJ Watson. Oxford University Press. In press.

24. Sakkas D, Lane and **Gardner DK** (2003) Assessment of embryo development and viability. In vitro fertilization and embryo recovery. In *A Laboratory Guide to the Mammalian Embryo* .eds. DK Gardner, M Lane and AJ Watson. Oxford University Press. In press.

25. Lane M, Baltz J and **Gardner DK** (2003) Analysis of intracellular ions: pH and calcium. In vitro fertilization and embryo recovery. In *A Laboratory Guide to the Mammalian Embryo* .eds. DK Gardner, M Lane and AJ Watson. Oxford University Press. In press.

26. **Gardner DK** and Lane M (2003) Nutrient uptake and metabolite production and enzyme activity and regulation. In vitro fertilization and embryo recovery. In *A Laboratory Guide to the Mammalian Embryo* .eds. DK Gardner, M Lane and AJ Watson. Oxford University Press. In press.

27. **Gardner DK** and Lane M (2003) Blastocyst metabolism. In *An Atlas of Human Blastocysts.* Eds. LL Veeck and N Zaninovic. Parthenon Publishing. pp 41-60.

Publications in Refereed Journals

1. **Gardner DK** and Leese HJ (1986) Non-invasive measurement of nutrient uptake by single cultured preimplantation mouse embryos. Hum. Reprod. 1: 25-27.

2. **Gardner DK** and Leese HJ (1987) Assessment of embryo viability prior to transfer by the noninvasive measurement of glucose uptake. J. exp. Zool. 242: 103-105.

3. **Gardner DK** and Leese HJ (1988) The role of glucose and pyruvate transport in regulating nutrient utilization by preimplantation mouse embryos. Development, 104: 423-429.

4. **Gardner DK**, Clarke RN, Lechene CP and Biggers JD (1989) Development of a noninvasive ultramicrofluorometric method for measuring net uptake of glutamine by single preimplantation mouse embryos. Gamete Res. 24: 427-438.

5. **Gardner DK** and Leese HJ (1990) Concentrations of nutrients in mouse oviduct fluid and their effects on embryo development and metabolism in vitro. J. Reprod. Fert. 88: 361-368.

6. Batt PA, **Gardner DK** and Cameron AWN (1991) Oxygen concentration and protein source affect the development of preimplantation goat embryos in vitro. Reprod. Fert. Devel. 3: 601-607.

7. Lane M and **Gardner DK** (1992) Effect of incubation volume and embryo density on the development and viability of mouse embryos in vitro. Hum. Reprod. 7: 558-562.

8. Weiss TJ, Warnes GM and **Gardner DK** (1992) Mouse embryos and quality control in human IVF. Reprod. Fert. Devel. 4:105-107.

9. Nichol R, Hunter RHF, **Gardner DK,** Leese HJ and Cooke GM (1992) Concentrations of energy substrates in oviductal fluid and blood plasma of pigs during the peri-ovulatory period. J. Reprod. Fert. 96: 699-707.

10. **Gardner DK** and Lane M (1993) Amino acids and ammonium regulate mouse embryo development in culture. Biol. Reprod. 4: 377-385.

11. **Gardner DK** and Sakkas D (1993) Mouse embryo cleavage, metabolism and viability: role of medium composition. Hum. Reprod. 8: 288-295.

12. **Gardner DK**, Lane M and Batt PA (1993) Uptake and metabolism of pyruvate and glucose by individual preattachment sheep embryos developed in vivo. Molec. Reprod. Devel. 36: 313-319.

13. Trounson A, Pushett D, Maclellan LJ, Lewis I and **Gardner DK** (1994) Current status of IVM/IVF and embryo culture in humans and farm animals. Theriogenology 41: 57-66.

14. **Gardner DK**, Lane M, Spitzer A and Batt PA (1994) Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage in vitro in the absence of serum and somatic cells: amino acids, vitamins and culturing embryos in groups stimulate development. Biol. Reprod. 50: 390-400.

15. Tsai F and **Gardner DK** (1994) Nicotinamide, a component of complex culture media, inhibits mouse embryo development in vitro and reduces subsequent developmental potential after transfer. Fertil. Steril. 61: 376-382.

16. Lane M and **Gardner DK** (1994) Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. J. Reprod. Fert. 102: 305-312.

17. **Gardner DK** (1994) Mammalian embryo culture in the absence of serum or somatic cell support. Cell Biol. International 18: 1163-1179.

18. Lane M and **Gardner DK** (1995) Removal of embryo-toxic ammonium from the culture medium by in situ enzymatic conversion to glutamate. J. exp. Zool. 271: 356-363.

19. Thompson JG, **Gardner DK**, Pugh PA, McMillan J and Tervit RH (1995) Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. Biol. Reprod. 53: 1385-1391.

20. Barnes FL, Crombie A, **Gardner DK**, Kausche A, Lacham-Kaplan O, Suikkari A-M, Tiglias J, Wood C and Trounson A (1995) Blastocyst development and birth after in-vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum. Reprod.10: 3243-3247.

21. Spindler RE, Renfree MB and **Gardner DK** (1995) Metabolic assessment of wallaby blastocysts during embryonic diapause and subsequent reactivation. Reprod. Fertil. Dev. 7: 1157-1162.

22. **Gardner DK**, Lane M, Calderon I and Leeton J (1996) Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. Fertil. Steril. 65: 349-353.

23. Leppens G, **Gardner DK** and Sakkas D (1996) Coculture of 1-cell outbred mouse embryos on bovine kidney epithelial cells: effect on development, glycolytic activity, inner cell mass:trophectoderm ratios and viability. Hum. Reprod.11: 598-603.

24. **Gardner DK**, Selwood L and Lane M (1996) Nutrient uptake and culture of Sminthopsis macroura (Stripe-faced dunnart) embryos. Reprod. Fert. Dev. 8: 685-690.

25. **Gardner DK**, Pawelczynski M and Trounson A (1996) Nutrient uptake and utilization can be used to select viable day 7 bovine blastocysts after cryopreservation. Mol. Reprod. Dev. 44: 472-475.

26. Lane M and **Gardner DK** (1996) Selection of viable mouse blastocysts prior to transfer using a metabolic criterion. Hum. Reprod., 11: 1975-1978.

27. Spindler RE, Renfree MB and **Gardner DK** (1996) Carbohydrate uptake by quiescent and reactivated mouse blastocysts. J. exp Zool. 276: 132-137.

28. **Gardner DK** and Lane M (1996) Alleviation of the '2-cell block' and development to the blastocyst of CF1 mouse embryos: role of amino acids, EDTA and physical parameters. Hum. Reprod. 11: 2703-2712.

29. Edwards LE, Batt PA, Gandolfi F and **Gardner DK** (1997) Modifications made to culture medium by bovine oviduct epithelial cells: changes to carbohydrates stimulate bovine embryo development. Molec. Reprod. Devel. 46: 146-154.

30. Lane M and **Gardner DK** (1997) Differential regulation of mouse embryo development and viability by amino acids. J. Reprod. Fertil. 109: 153-164.

31. Lane M and **Gardner DK** (1997) Nonessential amino acids and glutamine decrease the time of the first three cleavage divisions and increase compaction of mouse zygotes in vitro. J. Ass. Reprod. Genet. 14: 398-403.

32. **Gardner DK** and Lane M (1997) Culture and selection of viable blastocysts: a feasible proposition for human IVF? Hum. Reprod. Update 3: 367-382.

33. **Gardner DK**, Vella P, Lane M, Wagely L, Schlenker T and Schoolcraft WB (1998) Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertil. Steril. 69: 84-88.

34. **Gardner DK** (1998) Changes in requirements and utilization of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. Theriogenology 49: 83-102.

35. Jones GM, Trounson AO, **Gardner DK**, Kausche A, Lolatgis N and Wood C (1998) Evolution of a culture protocol for successful blastocyst development and pregnancy. Hum. Reprod. 13: 169-177.

36. Lane M and **Gardner DK** (1998) Amino acids and vitamins prevent culture-induced metabolic perturbations and associated loss of viability of mouse blastocysts. Hum. Reprod. 13: 991-997.

37. **Gardner DK** and Lane M (1998) Culture of viable human blastocysts in defined sequential serum-free media. Hum. Reprod. 13: Supplement 3: 148-159.

38. **Gardner DK** (1998) Development of serum-free media for the culture and transfer of human blastocysts. Hum. Reprod. 13: Supplement 4: 218-225.

39. **Gardner DK** and Schoolcraft WB (1998) Human embryo viability: what determines developmental potential and can it be assessed? J. Ass. Reprod. Genet. 15: 455-458.

40. Edwards LE, Williams DA and **Gardner DK** (1998) Intracellular pH of the preimplantation mouse embryo: Effects of extracellular pH and weak acids. Mol. Reprod. Devel. 50: 434-442.

41. Spindler RE, Renfree MB and **Gardner DK** (1998) Reactivating tammar wallaby blastocysts oxidize glucose Biol. Reprod. 58: 1425-1431.

42. **Gardner DK**, Schoolcraft WB, Wagley L, Schlenker T, Stevens J and Hesla J (1998) A prospective randomized trial of blastocyst culture and transfer in in vitro fertilization. Hum. Reprod.13: 3434-3440.

43. **Gardner DK** and Schoolcraft WB (1998) No longer neglected: the human blastocyst. Hum. Reprod.13: 3289-3292.

44. Edwards LE, Williams DA and **Gardner DK** (1998) Intracellular pH of the preimplantation mouse embryo: amino acids act as buffers of intracellular pH. Hum. Reprod. 13: 3441-3448.

45. Nichol R, Hunter RH, **Gardner DK**, Partridge R, Leese HJ, Cooke GM (1998) Concentrations of energy substrates in oviduct fluid in unilaterally ovariectomised pigs. Res. Vet. Sci. 65: 263-4.

46. Spindler RE, Renfree MB and **Gardner DK** (1999) Mouse embryos used as a bioassay to determine control of marsupial embryonic diapause. J. exp. Zool. 283: 590-9.

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131. Stilley KS, Lane M, **Gardner DK** (2003) Heterologous coculture of bovine embryos with mouse embryos stimulates blastocyst development and differentiation. Theriogenology, 59: 459.

INVITED CONFERENCE LECTURES / SEMINARS

1986	CIBA Foundation, London
1987	MRC Mammalian Development Unit, London
1988	Albert Einstein Medical Centre, Philadelphia
1988	Johns Hopkins Medical School, Baltimore
1989	Organon Embryology Symposium, Canberra
1990	IVF America Ltd, New York
1991	Primate Centre, Portland, Oregon
1991	IVF America Ltd, New York
1991	Centre for Early Human Development, Monash University, Melbourne
1992	Clinique de Fertilite et Sterlite, Universitaire de Geneva, Geneva
1992	Department of Obstetrics and Gynaecolgy, National University of Singapore
1993	AgResearch, Ruakura Agricultural Centre, New Zealand
1993	Serono Co-culture Symposium, Sydney
1993	Institute of Reproduction and Development, Monash University, Melbourne
1994	La Trobe University, Melbourne
1994	University of California, San Francisco
1994	IVF America Ltd, New York and Boston
1994	New York Academy of Science
1994	Plenary Lecture to the Fertility Society of Australia, Brisbane
1995	Boden Conference, Thredbo
1995	Serono Embryo Symposium, New Zealand Infertility Society, Christchurch
1995	Center for Reproductive Medicine, Denver
1996	North California Association of Reproductive Biologists, Stanford University
1996	Organon Symposium on Assisted Reproduction, Chicago
1997	Department of Obstetrics and Gynaecolgy, National University of Singapore
1997	10th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara
1997	Graduate Course at the American Society for Reproductive Medicine, Cincinnati
1997	Alpha / Serono Symposium on the Human Preimplantation Embryo, Sorrento, Italy
1997	Serono International Symposium on ART: State of the Art, Maui, Hawaii
1998	International Embryo Transfer Society, Boston
1998	IVF Hot Topics, Chicago
1998	Organon Symposium on ART in 21st Century, Chicago
1998	11th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara
1998	5th International Symposium on Reproduction in Domestic Ruminants, Colorado Springs
1998	Post Graduate Course at the American Society for Reproductive Medicine, San Francisco
1998	Society for the Study of Fertility / German Fertility Society, Aachen, Holland
1999	Pacific Coast Reproductive Society, San Diego
1999	Plenary Lecture at the World Congress on IVF and ART, Sydney
1999	12th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara
1999	St. Luke's Hospital, Oita, Japan
1999	World Congress on Obstetrics and Gynecology, Prague, Czech Republic
2000	British Fertility Society, Bath, UK
2000	13th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara

2000	ART and the Human Blastocyst, Serono Symposium, Dana Point
2000	Post Graduate Course at the American Society for Reproductive Medicine, San Diego
2001	Department of OBGYN, Louisville, Kentucky
2001	Human Embryo Implantation, Madrid, Spain
2001	14th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara
2001	World Congress on Obstetrics and Gynecology, Paris, France
2001	Post Graduate Course at the American Society for Reproductive Medicine, Orlando
2002	Biotechnology of Human Reproduction, Turin, Italy
2002	Gordon Research Conference, New London, CT
2002	Implantation: From Bench to the Bedside, Serono Symposium, Mohegan Sun, CT
2002	15th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara
2002	University of Zhongshan, Ghaungzhou, China
2002	University of Wuhan, China
2002	University of Beijing, China
2002	University of Shanghai, China
2003	Bertarelli Foundation, New York
2003	Royal Society of Medicine, London
2003	16th Annual In Vitro Fertilization and Embryo Transfer Update, Santa Barbara